

Stable Carbon Isotope Ratios for Detecting Added Sugars in Orange and Apple Juices and Added Citric Acid in Lemon Juices

L. W. DONER

1 Introduction

The adulteration of many foods, including the fruit juices, with relatively inexpensive substitutes, has long been common practice in many parts of the world. Such undeclared additions are especially prevalent during periods of high demand, when there may not be adequate supplies of the food. Substantial financial gains are realized by manufacturers who substitute an inexpensive ingredient for part or all of a food. In most countries, laws prohibit such practices, and when the food is appropriately labeled as having added ingredients, there is no legal violation. Widespread mislabeling, however, seriously affects markets for the food and constitutes a fraud to the consumer.

The juice market expanded rapidly after the development of frozen concentrated juices in 1946. Adulterated juices began to appear a short time later, when the considerable markets for juices were recognized. Limited supplies were extended by adding sugars, organic acids, and colorants, and even by adding less expensive juices, such as grapefruit juice to orange juice. These practices continue to this day, despite the considerable research efforts undertaken to combat them.

Early forms of adulteration were relatively unsophisticated, and the adulterant would most often contain a substance not normally present in the pure juice, but detectable in adulterated products. For the sophisticated adulterations practiced at the present time, this approach most often is not successful. Nor is the approach of attempting to identify a constituent or property in pure juices that is constant, and measuring its diminution upon adulteration. The wide variability among the known constituents of most pure foods precludes this approach.

This report is concerned with detecting a major form of juice adulteration, the addition of syrups derived from sugar cane and corn. These products, cane invert syrup and high fructose corn syrup (HFCS), are very inexpensive compared to orange and apple juices. Also, they are commercially available with sugar ratios within the range normally found in pure juices. So they would appear to be ideal adulterants, with their detection requiring the identification of subtle differences between them and pure juices. They can presently be detected by measurement of ratios of ($^{13}\text{G}/^{12}\text{C}$) in total carbon. These adulterants have a significantly higher ratio than do either orange or apple juice, as measured by stable isotope ratio mass spectrometry (SIRA). Similarly, citric acid, often used to adulterate lemon juice, has an elevated ($^{13}\text{C}/^{12}\text{C}$) ratio when it is produced by fermentation of cane or corn-derived sugar.

Some of the processes which are responsible for natural variations in ($^{13}\text{C}/^{12}\text{C}$) ratios will be described. This will enable the reader to envisage future

applications of the approach, and to predict when the approach may not be successful. Most responsible for variations in ($^{13}\text{C}/^{12}\text{C}$) ratios among plants and their derived products are the initial photosynthetic enzymes, ribulose biphosphate carboxylase ($\text{RuBP}_{\text{case}}$) and phosphoenolpyruvate carboxylase (PEP_{case}).

2 Photosynthetic Fractionation of Carbon Isotopes in Source CO_2

The ratio of ($^{13}\text{C}/^{12}\text{C}$) is very close to 1/90, but this ratio was first observed (Nier and Gulbransen 1939) to vary slightly, depending on the source of the carbon analyzed. Carbon in sources such as limestones, atmospheric carbon dioxide, marine plants, and land plants were found to possess characteristic ($^{13}\text{C}/^{12}\text{C}$) ratios.

Table 1. Natural abundance of stable isotopes applied to problems in food adulteration

Isotope	Natural abundance (atom %)
H	99.983
D	0.014
[^{12}C]	99.916
[^{13}C]	1.084
[^{16}O]	99.758
[^{18}O]	0.204

Variability in the relative abundance of isotopes exists for all elements, although tabulated values suggest that they are constant. Tabulated values for stable isotopes, which have been applied to detecting food adulteration problems, are given in Table 1. The differences in ($^{13}\text{C}/^{12}\text{C}$) ratios were elaborated upon in a survey of ratios in hundreds of samples from a variety of sources (Craig 1953; Wickman 1952), using a precise isotope ratio mass spectrometer. It was clear that the step most responsible for variations in ($^{13}\text{C}/^{12}\text{C}$) ratios in carbon-containing materials is the photosynthetic fixation of CO_2 by plants. All plants have lower ratios than does atmospheric CO_2 , but the magnitude of this difference from source CO_2 varied among plants. Explaining these observations that different types of plants have different ($^{13}\text{C}/^{12}\text{C}$) ratios awaited further understanding of photosynthetic differences among plants. A comprehensive review of carbon isotope fractionation in plants has been published (O'Leary 1981).

2.1 Instrumentation and the Concept of [$\delta\text{-}^{13}\text{C}$] Values

Isotope ratio mass spectrometers differ from other types in a variety of ways, all of which contribute to its sensitivity for determining very small differences in isotope enrichment [(< 0.00001) atom % excess]. Modern instruments are dual-inlet

and dual-collecting and are microcomputer controlled, allowing automatic operation. The dual inlet system allows measuring the isotope ratio of the sample gas against that of a reference gas containing a known isotope ratio. Measurements are made of small molecules, such as H_2 , N_2 , and CO_2 , and enrichments in minor isotopes of these gases can be determined. To analyze CO_2 , for example, the ion beams from $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ are collected by means of a double exit slit and are amplified simultaneously by a pair of matched amplifiers. The larger (representing mass 44) of the two amplified currents is then attenuated until it exactly balances the smaller (representing mass 45) current from the other amplifier. The isotope ratio in the reference (standard) gas is also measured, by periodically switching the dual-inlet system, and values in the sample are reported relative to those in the standard. Corrections are made for certain variables, such as the contribution of ^{17}O to the mass 45 signal. Since laboratories throughout the world report values relative to the same standards, agreement is generally excellent.

To prepare samples for isotope ratio analysis, it is essential that the element of interest be quantitatively converted to the gaseous form to be analyzed in the mass spectrometer. For ($^{13}\text{C}/^{12}\text{C}$) analysis of plant materials, for example, this is achieved by combusting several mg of sample in purified O_2 at about 800–900° C and then recirculating the gases over hot CuO . The products, H_2O and CO_2 , are frozen and then the CO_2 is distilled to a sample flask for analysis. Efficient sample preparation systems, as well as stable isotope ratio mass spectrometers, are commercially available.

Stable isotope ratios of carbon and other elements are reported in terms of a δ value. This value indicates the difference in parts per thousand (per mil) between the ratio of the sample relative to that in the standard. For ($^{13}\text{C}/^{12}\text{C}$) ratios, the standard is CO_2 prepared from a fossil skeleton from the Pee Dee formation of South Carolina, and [$\delta\text{-}^{13}\text{C}$] values are reported relative to PDB. The value [$\delta\text{-}^{13}\text{C}$] is defined as

$$[\delta\text{-}^{13}\text{C}] \text{ (per mil)} = [(^{13}\text{C}/^{12}\text{C})_{\text{sample}} / (^{13}\text{C}/^{12}\text{C})_{\text{PDB}} - 1] 10^3$$

The reproducibility of this measurement is ± 0.2 per mil or better. A value of +25 means that the ($^{13}\text{C}/^{12}\text{C}$) ratio of the sample is greater than that of the standard by 25 per mil or 2.5%. Conversely, a value of –25 means that the ratio of the sample is less than that of the standard by 25 per mil or 2.5%.

In the following discussions it will be apparent that carbon from plant materials invariably possesses negative [$\delta\text{-}^{13}\text{C}$] values, as do most organic carbons. It is the magnitude of this value that has been very useful in investigations, such as those on adulteration of foods.

2.2 C_3 (Calvin) Plants

The elucidation of a pathway by which plants reduce CO_2 to form sugar by Calvin's groups in the 1950's (Calvin and Bassham 1962) was long thought to include the only process for CO_2 fixation by plants. This pathway is commonly referred to as the C_3 cycle, since the initial product is a C-3 compound, 3-phosphoglyceric acid (PGA). It is produced by addition of atmospheric carbon dioxide to ribulose

bisphosphate (RuBP) in plant tissue, a reaction catalyzed by RuBP_{case}. Comprehensive surveys (Bender 1971; Smith and Epstein 1971) have established that all C₃ plants possess [δ -¹³C] values from about -22 to -33 per mil. So C₃ plants are depleted in ¹³C compared to the atmospheric CO₂ they utilize, which has a value of -7 per mil. The major factor responsible for C₃ plants being depleted in ¹³C relative to source is an isotope effect associated with the initial CO₂ fixation reaction (Park and Epstein 1960, 1961). Detailed in vitro studies have established that this discrimination against ¹³CO₂ is to the extent of 29 per mil (Roeske and O'Leary 1984). Many plant samples from the surveys and the earlier one by Craig (1953), however, had [δ -¹³C] values much closer to that of atmospheric carbon dioxide. This anomaly could be rationalized by additional in vitro studies after another pathway for CO₂ fixation was discovered in the 1960's.

2.3 C₄ (Hatch-Slack) Plants

In studies with sugar cane, it was observed that the C-4 acid oxaloacetate, and not PGA, was the initial product of photosynthesis (Kortschak et al. 1965). Oxaloacetate is rapidly reduced to malate or transaminated to aspartate. Hatch et al. (1967) confirmed this finding and showed that malate was produced from oxaloacetate after its formation from CO₂ and phosphoenolpyruvate (PEP), a reaction catalyzed by PEP_{case}. They also noted that this pathway is common to many tropical grasses and some dicotyledonous plants. As a result, such plants were referred to as Hatch-Slack or C₄ plants. It was first noted by Bender (1968) that plants having this type of photosynthesis are substantially less enriched in the ¹³C isotope than C₃ plants. They have ¹³C values ranging from about -9 to -20 per mil, with most at the higher end of this range. For example, corn and sugar cane have values near -12 per mil.

It should be emphasized that the C₃ pathway is ultimately operative in all C₄ plants, as malate (or another C-4 acid) initially produced in the mesophyll, is transported to neighboring bundle sheath cells. There it is decarboxylated, and the CO₂ produced condensed with RuBP, initiating the C₃ cycle. In C₄ plants the characteristic fractionation of carbon isotopes by RuBP_{case} does not occur, because the assimilation of CO₂ in the bundle sheath cells is quantitative. Any differences in [δ -¹³C] values between source CO₂ and C₄ plants would have then been due to fractionation by PEP_{case}. This enzyme has been shown to exhibit little or no fractionation of isotopes (Reibach and Benedict 1977), so values in C₄ plants are quite close to that in atmospheric CO₂. A review discussing the various CO₂ fixation pathways has been published (Hatch and Slack 1970).

2.4 CAM (Crassulacean Acid Metabolism) Plants

Members of the plant family Crassulaceae possess an unusual modification of the C₄ pathway. All species of Cactaceae also appear to possess this modification, as do succulents from a number of other plant families. These plants were termed

Table 2. Summary of photosynthesis by higher plants

Light reaction:	Generation of ATP and NADPH in leaf chloroplasts, to provide the energy and reducing potential required for CO ₂ reduction to carbohydrate.
Dark reaction:	<p>C₃ (<i>Calvin</i>) cycle; ultimately operative in all plants and is initiated by; CO₂ + RuBP → two 3-phosphoglyceric acid</p> <p>C₄ and CAM (crassulacean acid metabolism) pathways have efficient means to move CO₂, chemically bound in either malic or aspartic, to C₃ cycle reaction sites.</p> <p><i>In C₄ plant</i> mesophyll cells, CO₂ + PEP → malate or aspartate CO₂ is released to C₃ cycle in bundle sheath cells.</p> <p><i>In CAM plants</i>, during the night malate is produced, CO₂ + PEP → malate and during the day, CO₂ + RuBP → two 3-phosphoglyceric acid</p> <p>This final C₃ reaction may use CO₂ derived from malate, or to a lesser extent, CO₂ from the atmosphere.</p>

crassulacean acid metabolism (CAM) plants because accumulation of massive quantities of organic acids was first observed in a species of Crassulaceae. Like C₄ species, CAM plants have the capacity to fix CO₂ by reaction with either RuBP or PEP. They are unique in that CO₂ addition to PEP (catalyzed by PEP_{case}), producing malic acid, occurs at night when their leaf stomata are open, rather than during the day. In daylight, the malic acid is decarboxylated, and the CO₂ released is fixed into the C₃ cycle by RuBP_{case}. So in CAM plants the CO₂ fixing carboxylases are temporally separated (night-day), while in C₄ plants they are spatially separated (mesophyll cells-bundle sheath cells).

During the night, carbon fixed into CAM plants has a rather high value of [δ-¹³C], similar to that in C₄ plants, because of the lack of isotope fraction by PEP carboxylase. During the day however, some stomatal opening and atmospheric CO₂ fixation may occur, allowing the isotope discrimination characteristic of RuBP_{case}. As a result of this variability in fixation modes, succulent species capable of CAM have [δ-¹³C] values ranging from about -9 to -27 per mil. The relative proportion of night and day CO₂ fixation is a major determinant of this value (Sternberg et al. 1984). The most important source of food among the CAM plants is pineapple, and its value of [δ-¹³C] ranges from -11.2 to -12.9 per mil (Doner et al. 1979; Krueger et al. 1986). An excellent discussion of carbon metabolism by plants, including discussion of carbon isotope fractionation, has been published (Ting 1982), and distinctions between the three categories are summarized in Table 2. .

2.5 Additional Factors Contributing to Plant [δ-¹³C] Values

The value of [δ-¹³C] in plant carbon is primarily due to differences in photosynthetic CO₂ fixation, and more recently explanations have been provided as to why

members of the same category possess a range of values. Obviously, there are factors involved in addition to the initial photosynthetic enzymes involved.

2.5.1 Carbon Dioxide Diffusional Effects on Plant $[\delta\text{-}^{13}\text{C}]$ Values

In studies of plants grown under controlled environmental conditions (Farquhar et al. 1982), it has been found that factors which modify the diffusion of atmospheric CO_2 into the leaf mesophyll contribute to $[\delta\text{-}^{13}\text{C}]$ variability. By determining the concentration of CO_2 in the atmosphere and in leaf intercellular spaces, C_a and C_i respectively, $[\delta\text{-}^{13}\text{C}]$ values can be predicted with a good degree of accuracy (assuming constants) according to the expression

$$[\delta\text{-}^{13}\text{C}]_{\text{plant}} = [\delta\text{-}^{13}\text{C}]_{\text{atmosphere}} - a - (b - a)(C_i/C_a)$$

where the constant a (4.4 per mil) is the diffusivity of $^{12}\text{CO}_2$ relative to $^{13}\text{CO}_2$ and b (29 per mil) is the isotopic discrimination by $\text{RuBP}_{\text{case}}$. So variables such as the plant's structure and growing conditions, which may effect CO_2 diffusion, will alter C_i/C_a and the $[\delta\text{-}^{13}\text{C}]$ value.

2.5.2 Variations Among Classes of Plant Compounds

Significant differences exist between $[\delta\text{-}^{13}\text{C}]$ values in the different classes of organic compounds which can be isolated from plants. Different plants contain different proportions of carbohydrates and lipids for example, and the lipids are relatively depleted in ^{13}C . So this will account for some of the variability in plants from the same photosynthetic category. In studies of photosynthetic microorganisms, it has been noted that amino acids from these organisms vary in $[\delta\text{-}^{13}\text{C}]$ value, and that in an individual amino acid, the carboxyl carbon is heavier (more ^{13}C) in carbon than the rest of the molecule (Abelson and Hoering 1961). A broader study comparing values in different classes of compounds from cotton (a C_3 plant) and sorghum (a C_4 plant) was then conducted (Whelan et al. 1970). Whole leaf tissue gave $[\delta\text{-}^{13}\text{C}]$ values of -10.9 and -27.0 in sorghum and cotton, respectively, and a range of values was found in each for various compound classes. The sugars, organic acids, and amino acids were richer in ^{13}C than more extensively reduced classes, such as the lipids and carotenoids. Isotope effects are exhibited during the various metabolic cycles and pathways leading to these various classes of compounds in plants. The variations are small, however, compared to those resulting from the initial CO_2 fixation reactions.

3 Food Applications of Source Variations in $[\delta\text{-}^{13}\text{C}]$ Values

The natural variations in $[\delta\text{-}^{13}\text{C}]$ values among plants have been used to provide information in many food and nutritional areas. For example, information regarding food sources used by animals (even prehistoric) can be gained by examining $[\delta\text{-}^{13}\text{C}]$ values in tissues. For example, from studies of values in milk and hair of grazing cattle, relative dietary contributions of C_3 and C_4 plants in pastures

were determined (Minson et al. 1975). Similarly, it has been determined that Europeans rely more on C_3 dietary sources than do Americans. This is primarily due to the fact that in the United States large amounts of the C_4 sources, corn and sorghum, are used as feeds in the pork, beef, poultry, and dairy industries, while in Europe C_3 feed sources are commonly used. As a result, tissue samples from people in New York and Lucerne averaged -16.5 and -21.0 per mil respectively (Gaffney et al. 1978). Stable isotope analysis of bone collagen has been widely used (van der Merwe 1982) to reconstruct the diets of prehistoric humans. An extensive study of values for $[\delta-^{13}C]$ and $[\delta-^{15}N]$ in collagen from human bones of various ages and from a variety of sites was recently described (DeNiro 1985). The use of isotopes in a variety of metabolic and nutritional investigations has been comprehensively reviewed (Matthews and Bier 1983).

In their survey of variations in $[\delta-^{13}C]$ values among plants (Smith and Epstein 1971), values were given for sugar cane (C_4 plant) and sugar beet (C_3 plant). These values were -13.9 and -30.1 per mil respectively, and the act of processing sources into foods results in minimal further fractionation of isotopes. So the authors suggested that one could easily distinguish between pure sucrose from these two plants. Chemically the sugars are identical of course, and this distinction would not be possible by other means. This concept provided the impetus for methods to be developed not only for detecting added sugars in foods, but also for detecting other illicit mixtures from C_3 and C_4 plant sources.

3.1 Detecting Mixtures of C_3 and C_4 Plant-Derived Foods

Application to foods of natural variations in stable carbon isotope ratios has been subject of previous reviews (Winkler and Schmidt 1980; Bricout 1982; Krueger 1984). In addition, a volume has included several chapters on this and related topics (Schmidt et al. 1982). Among the applications have been the detection of honey adulteration (Doner and White 1977), the differentiation of corn-fed animal protein from soy protein (Gaffney et al. 1979), the classification of vanillin as natural or synthetic (Hoffman and Salb 1979), the determination of CO_2 and ethanol origin in wines (Dunbar 1982), and the topic of this report, detecting adulteration in juices.

For foods such as honey, apple juice, and orange juice, ideal adulterants are inexpensive materials which contain the sugars fructose, glucose, and sucrose in proportions within the range normally found in these foods. Such is the case with cane and beet syrups, and high fructose corn syrup (HFCS). Prior to industrial development of methods to prepare fructose/glucose syrups from cane and beet sucrose by invertase hydrolysis, these syrups were quite easy to detect in adulterated foods. They had been prepared by acid hydrolysis of sucrose, and these syrups invariably contained high levels of hydroxymethylfurfural (HMF), which could be readily quantified and used as a marker for adulteration. In some parts of the world, invert syrups continue to be produced by acid hydrolysis of sucrose, so HMF measurement is still among the analytical strategies to detect adulteration.

The fructose/glucose syrups prepared by invertase hydrolysis of sucrose contain no such marker as HMF, nor do the similar syrups prepared by glucose

isomerase conversion of corn-derived glucose to HFCS. These syrups are highly refined, and their composition is simple compared to the juices from apple and citrus, which are extremely complex natural products. It is fortunate that sugar cane and corn are C_4 plants while the juices are derived from C_3 plants, since HFCS was introduced in the early 1970's and there was evidence that it and cane and beet invert syrups were being illegally added to these juices. At that point in time, it was quite predictable that by determining $[\delta-^{13}C]$ values, it would be possible to detect adulterated mixtures. Syrups derived from the C_3 plant sugar beet would be undetectable by this procedure, but other isotopic approaches would prove useful for their detection, and these will be mentioned in the Conclusion.

3.1.1 Cane and High Fructose Corn Syrups in Orange Juice

Some of the earliest applications of isotope ratio analysis to foods were by Bricout and his colleagues. In France, law distinguishes between natural fruit juices and diluted concentrates, and it was shown that the metabolic water in the natural juices contained higher ratios of D/H and ($^{18}O/^{16}O$) than did the groundwater used to reconstitute concentrates. This procedure was found useful for both apple (Bricout et al. 1973) and orange (Bricout 1973) juices.

The use of $[\delta-^{13}C]$ measurements to detect the addition of cane syrups to orange juice was demonstrated when values in genuine samples of French and Israeli juices were reported (Nissenbaum et al. 1974). Values of -25.0 and -24.3 per mil were obtained for French and Israeli orange juices respectively, and since cane sugar possessed a value of -12.2 per mil, mixtures could be detected. Further studies were initiated elsewhere, but in Israel, no application of this finding could be realized since beet sugar (-24.3 per mil) is used almost exclusively in Israel. From elevated δD and $[\delta-^{18}O]$ values in juices, however, addition of water could be detected.

A Canadian report (Hillaire-Marcel et al. 1977) on detecting the illegal addition of cane sucrose to maple syrup from $[\delta-^{13}C]$ analysis led us to assess the possibility of detecting honey adulteration by this means. Unlike foods such as citrus juices, wines, and maple syrups, known to be derived from C_3 plants, honey has its origin in the nectars from a number of plant families. A survey of $[\delta-^{13}C]$ values in honeys from all significant floral sources (Doner and White 1977; White and Doner 1978a) revealed that all did originate in C_3 plants. The range of $[\delta-^{13}C]$ values was -22.5 to -27.4 per mil, and if cane and corn derived syrups were added, these values were elevated. It could be predicted that mixtures of any C_3 and C_4 plant-derived foods would be detectable. The sensitivity of detection would depend on the natural variability in $[\delta-^{13}C]$ values for each component of the mixture.

Since it was likely that HFCS would be used by some manufacturers to adulterate orange juice, baseline $[\delta-^{13}C]$ values were measured in pure juices (Doner and Bills 1981). The values were as expected for C_3 plant-derived materials, and the range was quite narrow (-23.6 to -25.6 per mil) for 38 juices originating in the southern United States. The distribution of $[\delta-^{13}C]$ values of these samples is shown in Fig. 1, along with those of apple juice, source CO_2 , and the adulterants HFCS and cane syrup. The data represented in Fig. 1 suggest that HFCS and cane

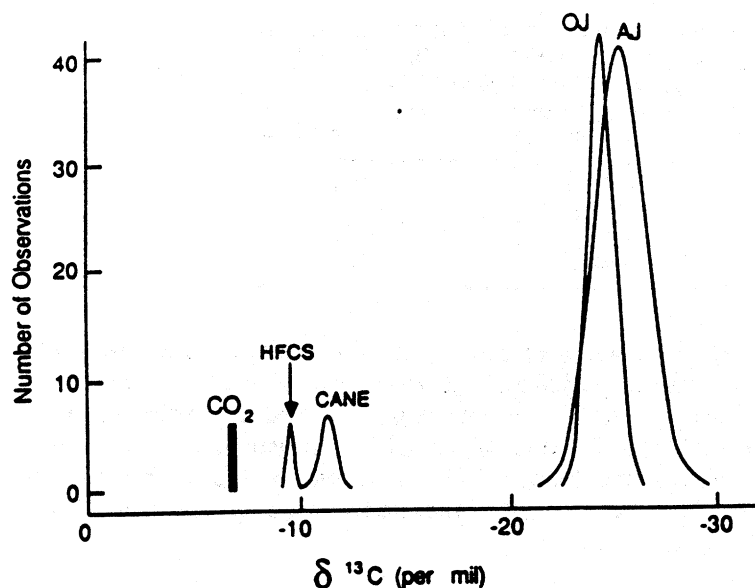


Fig. 1. Distribution in $[\delta^{13}\text{C}]$ values for atmospheric carbon dioxide, the adulterants cane and high fructose corn syrups, and pure orange and apple juices produced in the United States (Doner et al. 1980; Doner and Bills 1981)

syrups can be distinguished by $[\delta^{13}\text{C}]$ analysis, but this is not certain. Several orange juice samples from Mexico, Spain, and South Africa also had values in the range shown for orange juices. In order for the procedure to gain legal sanction, it was necessary to demonstrate that independent laboratories provided similar $[\delta^{13}\text{C}]$ values when analyzing a series of prepared adulterated mixtures, the contents of which were unknown to them. According to procedures of the Association of Official Analytical Chemists (AOAC), samples were prepared containing various proportions of orange juice and HFCS. Results from seven independent laboratories were in good agreement (Doner and Bills 1982), and the method was sanctioned as an official AOAC procedure, which is widely used by various regulatory agencies and industrial groups.

The $[\delta^{13}\text{C}]$ value in the pure juice component of a suspect product cannot be known because of the natural range in values, so the procedure is not quantitative. For example, if a juice with a value of -25.6 per mil was adulterated to the 50% level with HFCS at -9.8 per mil, the resulting mixture would give a value of -17.7 per mil. If a pure juice at the higher end of the range was adulterated to this level, an elevated $[\delta^{13}\text{C}]$ would result. Because of this uncertainty, the upper limit of $[\delta^{13}\text{C}]$ for pure juices is to be set with any desired degree of certainty as the mean value plus a multiple of the standard deviation for pure juices, as shown in Table 3 for orange and apple juices. Using this approach, there is only a 0.003% probability that a pure orange juice will be found with a $[\delta^{13}\text{C}]$ value less negative than -22.1 per mil, which is 4 standard deviation units from the mean for pure juices.

Table 3. Probabilities of pur orange and apple juices having values of $[\delta^{13}\text{C}]$ more negative than the stated limit (Doner and Phillips 1981; Doner and Bills 1982)

Probability %	Orange juice	Apple juice
84.10	-23.9	-24.1
97.72	-23.3	-22.8
99.87	-22.7	-21.5
99.997	-22.1	-20.2

All of the $[\delta^{13}\text{C}]$ measurements described above were conducted on CO_2 generated from total orange juice solids. It was subsequently shown (Parker 1982) that the sensitivity of the procedure can be enhanced for borderline samples with values near -23 per mil. Values of $[\delta^{13}\text{C}]$ were measured in the whole juice, pulp, and the soluble fraction. Since HFCS would reside in the soluble fraction, differences between $[\delta^{13}\text{C}]$ values in this fraction and the pulp (juice source) would suggest adulteration.

3.1.2 Cane and High Fructose Corn Syrups in Apple Juice

Surveys of $[\delta^{13}\text{C}]$ values in market apple juices in 1978 suggested that as much as one-third of the juice in New England was adulterated, probably with HFCS. Values near -17.0 per mil were commonly encountered, suggesting adulteration near the 50% level. In order to determine the permissible upper limit in $[\delta^{13}\text{C}]$ for pure juices, baseline values for $[\delta^{13}\text{C}]$ were determined (Doner et al. 1980) for juices representing the important apple varieties and growing areas in the United States. The range in values was -22.5 to -27.9 per mil (see Fig. 1 for distribution of values), greater than that found in orange juices, but sufficiently narrow to allow HFCS detection. The mean value for the 40 samples was -25.3 per mil, and the standard deviation 1.275. So, using the procedure described above for orange juice, the recommended cutoff for pure juices was set at $[-25.3 \text{ per mil} + 4(1.275) = -20.2 \text{ per mil}]$ (Table 3). Suspect juices with $[\delta^{13}\text{C}]$ values less negative than this would almost certainly be adulterated. A successful AOAC inter-laboratory study was conducted (Doner and Phillips 1981), wherein independent laboratories analyzed various apple juice-HFCS mixtures, and the method was officially sanctioned. In the United States, the procedure was used by the apple juice industry in self-policing efforts, and instances of adulteration with HFCS were markedly reduced.

3.1.3 Citric Acid in Lemon Juice

While syrups derived from sugar cane and corn may be added to maintain Brix acid ratios in adulterated lemon juices, the primary adulterant is inexpensive citric acid. This acid accounts for over 60% of the soluble solids in lemon juice, and many years ago, lemons were used as the source for commercial citric acid. At the present time, it is produced by microbial fermentation of several materials, including beet and cane molasses, corn sugar, and more recently, paraffin hydrocar-

bons. It could be assumed that citric acid prepared from cane and corn sugars would possess values of $[\delta^{13}\text{C}]$ much higher than those in lemon-derived citric acid. The acid, isolated from lemons after conversion to its calcium salt, possessed a mean value in $[\delta^{13}\text{C}]$ of -23.8 per mil, while the commercial samples from beet sugar, corn sugar, and paraffin hydrocarbon fermentation possessed values of -25.2 , -9.9 , and -27.7 per mil, respectively (Doner 1985). As a result, that from corn sugar would be readily detected, that from beet sugar would not be detected, and that from paraffin hydrocarbons would probably not be detected unless a very high proportion was added to lemon juice.

Petroleum products such as paraffin are devoid of ^{14}C , so it is likely that adulteration of lemon juice with citric acid derived from paraffin could be detected by scintillation counting of this radioactive isotope. This approach has been successfully applied to detecting synthetic alcohol in wines and spirits (McWeeny and Bates 1980) and for distinguishing natural and synthetic cinnamic aldehyde (Hoffman and Salb 1980). If sugars derived from C_4 plant sources were added to maintain the Brix acid ratio appropriate for lemon juice, they can be detected by determining $[\delta^{13}\text{C}]$ values after removal of citric acid as its calcium salt (Doner 1985).

4 Conclusion

It has been the intent of this review to provide theoretical bases for variations in ($^{13}\text{C}/^{12}\text{C}$) ratios among plants and to offer examples of how these variations can be exploited to combat some significant forms of food adulteration. Contemporary adulterative strategies are sophisticated, and conventional approaches such as identifying a constituent unique to the adulterant are most often unrewarding.

Table 4. $[\delta^{13}\text{C}]$ values in various fruit juices and sugars

Sample (#)	Photosynthesis category	Mean per mil	Range per mil	Std. Dev.
<i>Juice</i>				
Orange ^a (42)	C_3	-24.5	-23.4 to -25.6	0.591
Lemon ^{b,c} (5)	C_3	-24.6	-23.6 to -26.2	—
Lime ^a (3)	C_3	-24.3	-23.7 to -24.7	—
Grapefruit ^a (3)	C_3	-26.1	-24.9 to -26.8	—
Apple ^d (41)	C_3	-25.3	-22.5 to -27.9	1.275
Pineapple ^{b,c} (4)	CAM	-12.2	-11.2 to -12.9	—
<i>Sugar</i>				
Cane ^{e,f} (7)	C_4	-11.3	-10.5 to -12.2	—
Corn ^h (4)	C_4	-9.7	-9.5 to -9.8	—
Beet ⁱ (3)	C_3	-25.4	-25.1 to -26.0	—

^a Doner and Bills (1981).

^b Doner (1985).

^c Krueger et al. (1986).

^d Doner et al. (1980).

^e Doner et al. (1979).

^f Bricout and Fontes (1974).

^g Smith (1975).

^h White and Doner (1978b).

Instead, it is necessary to identify more subtle differences between food and adulterant, such as those described in this report.

To summarize, plants which utilize the C_3 pathway of photosynthetic CO_2 fixation are depleted in ^{13}C when compared to those which utilize the C_4 pathway. The difference between carbon in C_3 and C_4 plants is on the order of 15 per mil, a difference easily measured by stable isotope ratio mass spectrometry. Most of the difference is accounted for by an isotope effect exhibited by ribulose biphosphate carboxylase, the CO_2 fixing enzyme in C_3 plants. The differences in $[\delta-^{13}C]$ values between C_3 and C_4 plants is maintained throughout processing to foods. As a result, success in detecting food adulteration is assured when it can be determined that the target for adulteration uses the C_3 pathway, and the adulterant uses the C_4 pathway, or vice versa. Over the past 15 years, the approach has been successfully applied to combat adulteration of several foods, including fruit juices. In Table 4 the values of $[\delta-^{13}C]$ for several juices and adulterants are summarized, and it is clear that photosynthetic category is the primary determinant of this value.

4.1 Potential Future Applications of Isotope Ratios

When a food is suspected to be a target for adulteration, the value of employing $[\delta-^{13}C]$ measurements can often be determined by consulting the reviews of values in source plants (Craig 1953; Bender 1971; Smith and Epstein 1971). $[\delta-^{13}C]$ values in many edible fruits have been surveyed (Krueger et al. 1986), and only pineapple and prickly pear (CAM sources) were not derived from C_3 plants.

In locations where adulterations of juices with sugars from C_4 sources have been discouraged by application of ($^{13}C/^{12}C$) detection methods, adulteration with beet sugar has become widespread. Being from a C_3 plant source, it has been undetectable using this approach. Natural variations in D/H ratios of carbon-bound atoms in sugars offer great promise for detecting beet sugar adulteration of C_3 plant-derived foods. For example, sugars from wines have significantly elevated D/H ratios when compared to those in beet sucrose (Dunbar et al. 1983). More recently, it has been shown that sugars from orange juice also possess higher D/H ratios in carbon-bound atoms than does beet sucrose (Bricout and Koziel 1987). In analyzing sucrose isolated from orange juice and beet syrups, both carbon-bound D/H and ($^{18}O/^{16}O$) ratios were found to be higher in orange juice (Doner et al. 1987). It can be anticipated that similar measurements will be applied to detecting other forms of food adulteration, and that variations in ratios of other isotopes, even of minerals, will be used.

References

- Abelson P, Hoering TC (1961) Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. *Proc Natl Acad Sci USA* 47:623-632
- Bender MM (1968) Mass spectrometric studies of carbon 13 variations in corn and other grasses. *Radiocarbon* 10:468-472

- Bender MM (1971) Variations in the $^{13}\text{C}/^{12}\text{C}$ ratios of plants in relation to the pathway of photosynthetic carbon dioxide fixation. *Phytochemistry* 10:1239-1244
- Bricout J (1973) Control of authenticity of fruit juices by isotopic analysis. *J Assoc Off Anal Chem* 56:739-742
- Bricout J (1982) Possibilities of stable isotope analysis in the control of food products. In: Schmidt HL, Forstel H, Heinzinger K (eds) *Stable isotopes*. Elsevier, Amsterdam, pp 483-493
- Bricout J, Fontes JC (1974) Distinction analytique entre sucre de canne et sucre de betterave. *Ann Fals Exp Chim* 716:211-215
- Bricout J, Koziet J (1987) Control of the authenticity of orange juices by isotopic analysis. *J Agric Food Chem* 35:158-160
- Bricout J, Merlivat L, Fontes JC (1973) Sur la composition en isotopes stables de l'eau des jus de pommes. *Ind Alim Agric* 90:19-22
- Calvin M, Bassham JA (1962) *The photosynthetic carbon compounds*. Benjamin, New York
- Craig H (1953) The geochemistry of the stable carbon isotopes. *Geochim Cosmochim Acta* 3:53-92
- DeNiro MJ (1985) Postmortem preservation and alteration of in vivo bone collagen isotope ratios in relation to paleodietary reconstruction. *Nature* 317:306-309
- Doner LW (1985) Carbon isotope ratios in natural and synthetic citric acid as indicators of lemon juice adulteration. *J Agric Food Chem* 33:770-772
- Doner LW, Bills DD (1981) Stable carbon isotope ratios in orange juice. *J Agric Food Chem* 29:803-804
- Doner LW, Bills DD (1982) Mass spectrometric $^{13}\text{C}/^{12}\text{C}$ determinations to detect high fructose corn syrup in orange juice: collaborative study. *J Assoc Anal Chem* 65:608-610
- Doner LW, Phillips JG (1981) Detection of high fructose corn syrup in apple juice by mass spectrometric $^{13}\text{C}/^{12}\text{C}$ analysis: collaborative study. *J Assoc Off Anal Chem* 64:85-90
- Doner LW, White JW, Jr (1977) Carbon-13/carbon-12 ratio is relatively uniform among honeys. *Science* 197:891-892
- Doner LW, Chia D, White JW, Jr (1979) Mass spectrometric $^{13}\text{C}/^{12}\text{C}$ determinations to distinguish honey and C_3 plant sirups from C_4 plant sirups (sugar cane and corn) in candied pineapple and papaya. *J Assoc Off Anal Chem* 62:928-930
- Doner LW, Krueger HW, Reesman RH (1980) Isotopic content of carbon in apple juice. *J Agric Food Chem* 28:362-364
- Doner LW, Ajie HO, Sternberg L, Milburn JM, DeNiro MJ, Hicks KB (1987) Detecting sugar beet syrups in adulterated orange juices by stable hydrogen and oxygen isotope ratio analysis of sucrose. *J Agric Food Chem* 35:610-612
- Dunbar J (1982) Use of $^{13}\text{C}/^{12}\text{C}$ ratios for studying the origin of CO_2 in sparkling wines. *Fres Z Anal Chem* 311:578-580
- Dunbar J, Schmidt HL, Woller R (1983) Möglichkeiten des Nachweises der Zuckering von Wein über die Bestimmung von Wasserstoff-Isotopenverhältnissen. *Vitis* 22:375-386
- Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Aust J Plant Physiol* 9:121-137
- Gaffney JS, Irsa AP, Friedman L, Slatkin DN (1978) Natural $^{13}\text{C}/^{12}\text{C}$ variations in human populations. *Biomed Mass Spectrom* 5:495-497
- Gaffney JS, Irsa AP, Friedman L, Emken E (1979) $^{13}\text{C}/^{12}\text{C}$ analysis of vegetable oils, starches, proteins, and soy-protein mixtures. *J Agric Food Chem* 27:475-478
- Hatch MD, Slack CR (1970) Photosynthetic CO_2 -fixation pathways. *Annu Rev Plant Physiol* 21:141-162
- Hatch MD, Slack CR, Johnson HS (1967) Further studies on a new pathway of photosynthetic carbon fixation in sugar-cane and its occurrence in other plant species. *Biochem J* 102:417-422
- Hillaire-Marcel C, Carro-Jost O, Jacob C (1977) Composition isotopique $^{13}\text{C}/^{12}\text{C}$ du saccharose et du glucose de diverses origines et contrôle de l'authenticité des Sirops et sucres d'érable. *J Inst Can Sci Technol Aliment* 10:333-335

- Hoffman PG, Salb M (1979) Isolation and stable isotope ratio analysis of vanillin. *J Agric Food Chem* 27:352-355
- Hoffman PG, Salb M (1980) Radiocarbon (^{14}C) method for authenticating natural cinnamic aldehyde. *J Assoc Off Anal Chem* 63:1181-1183
- Kortschak HP, Hartt CE, Burr GO (1965) Carbon dioxide fixation in sugarcane leaves. *Plant Physiol* 40:209-213
- Krueger DA, Krueger RG, Krueger HW (1986) Carbon isotope ratios of various fruits. *J Assoc Off Anal Chem* 69:1035-1036
- Krueger HW (1984) Detection of adulteration and fraud in food products using MS. *Am Lab* 1:90-91
- Matthews DE, Bier DM (1983) Stable isotope methods for nutritional investigation. *Annu Rev Nutrit* 3:309-339
- McWeeny DJ, Bates ML (1980) Discrimination between synthetic and natural ethyl alcohol in spirits and fortified wines. *J Food Technol* 15:407-412
- Minson DJ, Ludlow MM, Troughton JH (1975) Differences in natural carbon isotope ratios of milk and hair from cattle grazing tropical and temperate pastures. *Nature* 256:602
- Nier AO, Gulbransen EA (1939) Variations in the relative abundance of the carbon isotopes. *J Am Chem Soc* 61:697-698
- Nissenbaum A, Lifshitz A, Stepak Y (1974) Detection of citrus fruit adulteration using the distribution of natural stable isotopes. *Lebensm Wiss Unters Technol* 7:152-154
- O'Leary MH (1981) Carbon isotope fractionation in plants. *Phytochemistry* 4:553-567
- Park R, Epstein S (1960) Carbon isotope fractionation during photosynthesis. *Geochim Cosmochim Acta* 21:110-216
- Park R, Epstein S (1961) Metabolic fractionation of ^{13}C and ^{12}C in plants. *Plant Physiol* 36:133-138
- Parker PL (1982) The chemical basis of the use of $^{13}\text{C}/^{12}\text{C}$ ratios to detect the addition of sweeteners to fruit juice concentrates. *Flüssiges Obst* 12:672-673
- Reibach PH, Benedict CR (1977) Fractionation of stable carbon isotopes by phosphoenolpyruvate carboxylase from C_4 plants. *Plant Physiol* 59:564-568
- Roeske CA, O'Leary MH (1984) Carbon isotope effects on the enzyme-catalyzed carboxylation of ribulose biphosphate. *Biochemistry* 23:6275-6284
- Schmidt HL, Forstel H, Heinzinger K (eds) (1982) *Stable isotopes*. Elsevier, Amsterdam
- Smith BN (1975) Carbon and hydrogen isotopes of sucrose from various sources. *Naturwissenschaften* 62:390
- Smith BN, Epstein S (1971) Two categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant Physiol* 47:380-384
- Sternberg LO, DeNiro MJ, Ting IP (1984) Carbon, hydrogen, and oxygen isotope ratios of cellulose from plants having intermediary photosynthetic modes. *Plant Physiol* 74:104-107
- Ting IP (1982) *Plant physiology*. Addison-Wesley, Reading MA
- Van der Merwe NJ (1982) Carbon isotopes, photosynthesis and archeology. *Am Sci* 70:596-606
- Whelan T, Sackett WM, Benedict CR (1970) Carbon isotope discrimination in a plant possessing the dicarboxylic acid pathway. *Biochem Biophys Res Commun* 41:1205-1210
- White JW, Jr, Doner LW (1978 a) The $^{13}\text{C}/^{12}\text{C}$ ratio in honey. *J Apic Res* 17:94-99
- White JW Jr, Doner LW (1978 b) Mass spectrometric detection of high-fructose corn syrup in honey by use of $^{13}\text{C}/^{12}\text{C}$ ratio: collaborative study. *J Assoc Off Anal Chem* 61:746-750
- Wickman FE (1952) Variations in the relative abundance of the carbon isotopes in plants. *Geochim Cosmochim Acta* 2:243
- Winkler FJ, Schmidt HL (1980) Einsatzmöglichkeiten der ^{13}C -Isotopen-Massenspektrometrie in der Lebensmitteluntersuchung. *Z Lebensm Unters Forsch* 171:85-94